

COMPARISON OF GRADIENT SLOPE, COLUMN LENGTH, AND PARTICLE SIZES FOR ANALYSIS OF PEPTIDES ON A 10,000 PSI NANOLC/MS/MS PLATFORM

Jeffrey W. Finch, Hongji Liu, Keith Fadgen, Geoff Gerhardt, James P. Murphy, and John C. Gebler
Life Sciences R&D, Waters Corporation, Milford, MA, USA

OVERVIEW

Purpose:

- Directly compare performance of 75 μm i.d. nanocolumns packed with 1.7 μm versus 3 μm particles for peptide digest separations, investigating effects of column length and gradient length (slope) on peak capacity

Methods:

- 10,000 psi nanoACQUITY UPLC™ system coupled to a quadrupole time-of-flight (Q-ToF) instrument

Results:

- 1.7 μm particle columns yield higher overall peak capacity gains with column and gradient length vs. 3 μm particle columns
- Shorter (ex. 15 cm) 1.7 μm columns are capable of yielding higher efficiencies vs. longer (ex. 50 cm) 3 μm columns
- There is a tradeoff between increased peak capacity and slight reduction in MS signal intensity with longer gradient times

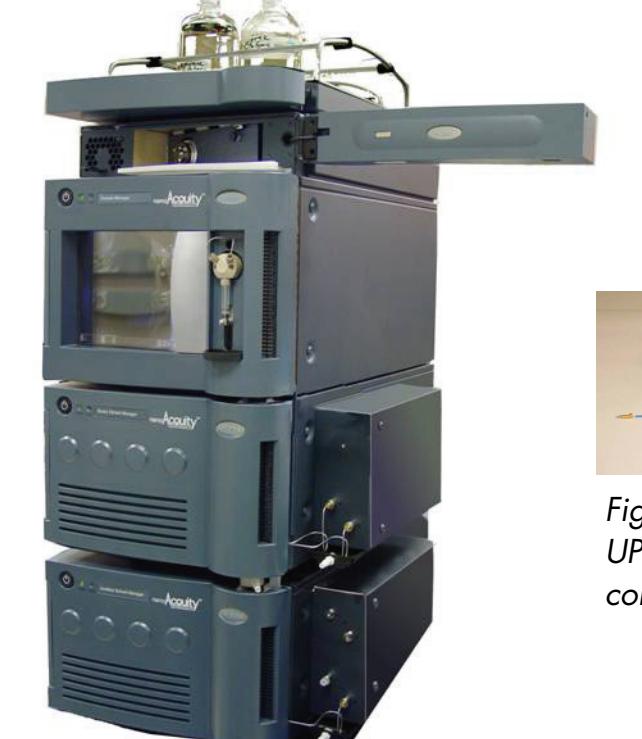


Figure 2. nanoACQUITY UPLC™ System

INTRODUCTION

The ability to increase nanoLC separation efficiency is highly desired for obtaining as much MS/MS information as possible where amounts of complex proteomics samples are often limited [1]. Methods for improving separation efficiency for typical reversed-phase nanoLC separations include using smaller particles, longer columns, and longer (more shallow) gradient times [2,3]. The use of smaller particles (< 2 μm) requires a nanoLC system capable of operating at higher backpressure for extended periods of time [4]. However, there have been few fundamental studies comparing effects of particle size, column length, and gradient length (time) on peak capacity, for nanocolumns where a) columns are prepared under very tightly-controlled conditions (same column assembly protocol, packing pressure, etc.), and b) columns are analyzed with a direct-flow commercially available nano UPLC system. In this presentation we directly compare effects of gradient slope, and column length for two different particles: a novel 1.7 μm bridged-ethyl hybrid (BEH) particle and a 3 μm particle.

METHODS

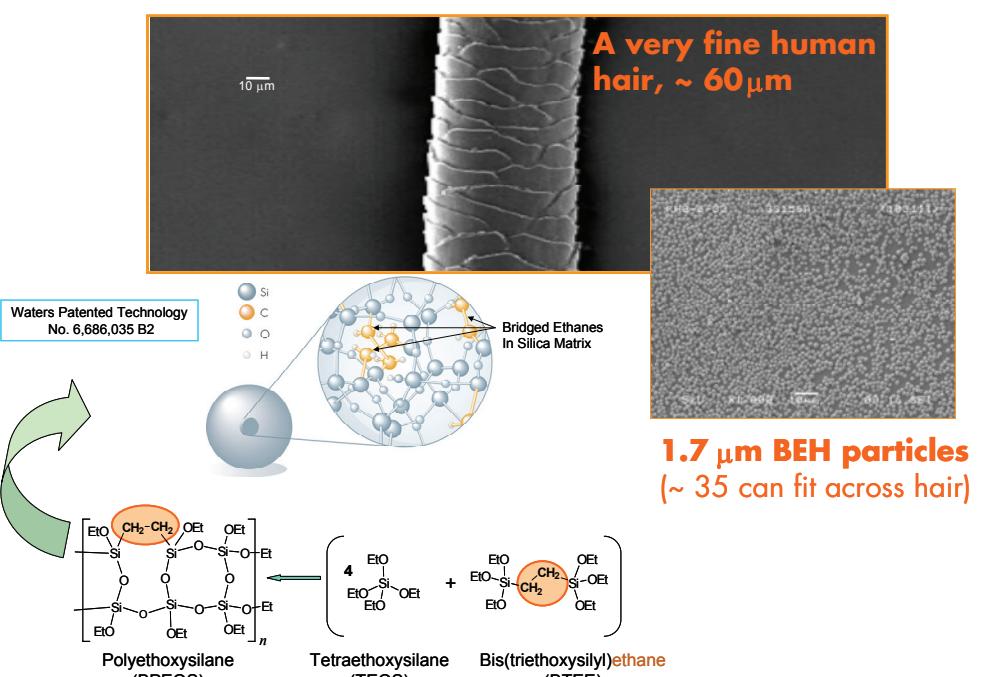


Figure 1. Bridged-ethyl hybrid (BEH) 1.7 μm particle chemistry

RESULTS

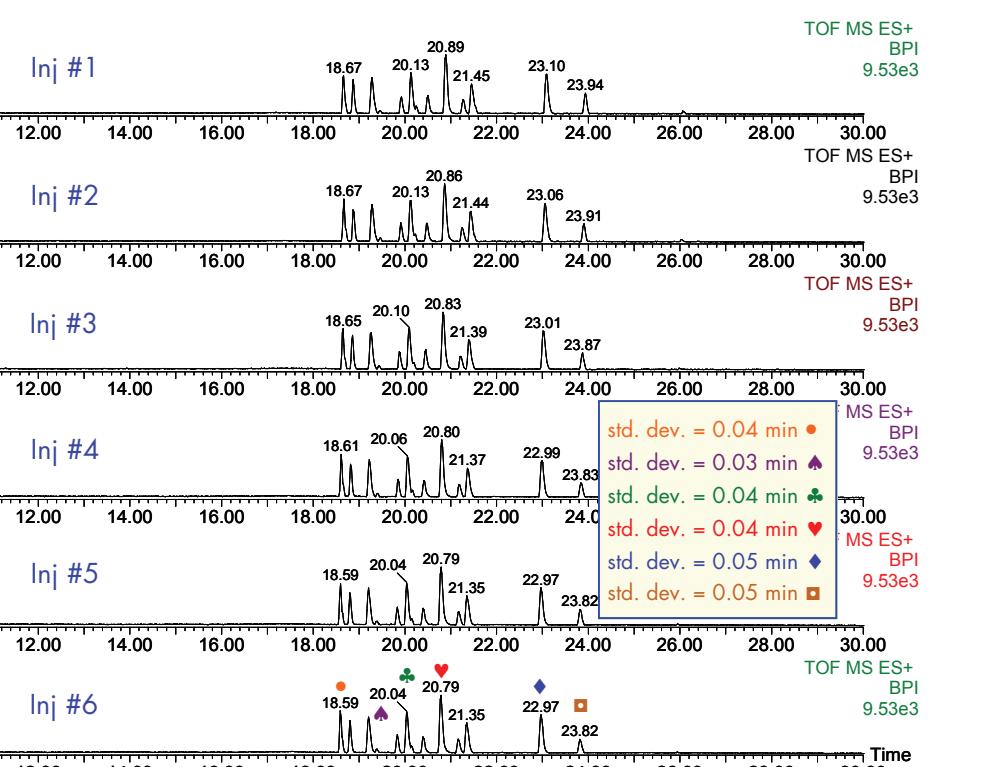


Figure 3. nanoACQUITY UPLC™ 75 μm x 25 cm column

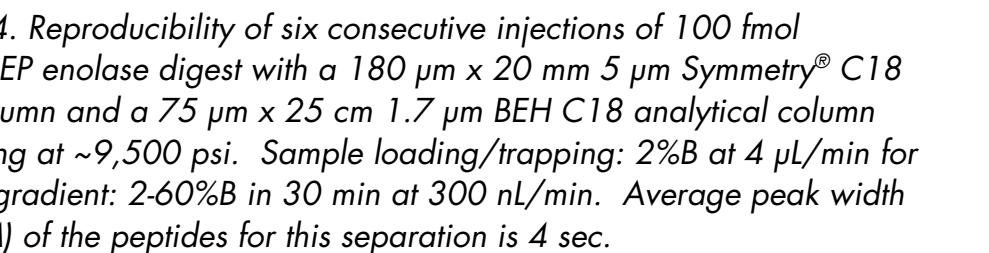


Figure 4. Reproducibility of six consecutive injections of 100 fmol MassPREP™ yeast enolase digest with a 180 μm x 20 mm 5 μm Symmetry® C18 trap column and a 75 μm x 25 cm 1.7 μm BEH C18 analytical column operating at ~9,500 psi. Sample loading/trapping: 2% B at 4 $\mu\text{l}/\text{min}$ for 3 min, gradient: 2-60% B in 30 min at 300 nL/min. Average peak width (FWHM) of the peptides for this separation is 4 sec.

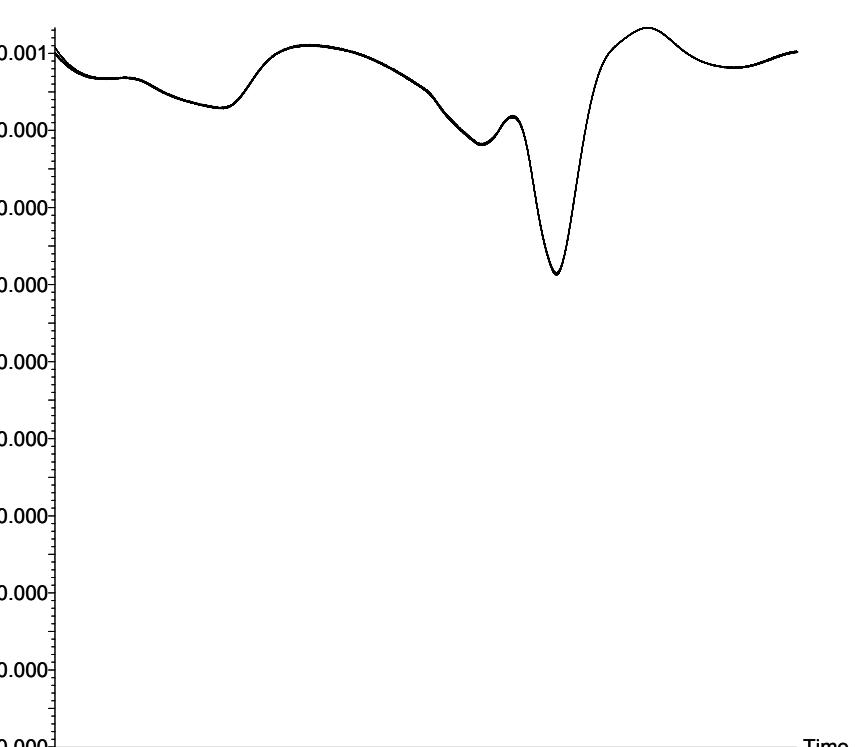


Figure 5. Overlay of pressure traces for six consecutive injections recorded on a nano UPLC system, for data shown in Figure 4. Note the excellent reproducibility of gradient delivery for multiple injections recorded over a period of 9 hours.

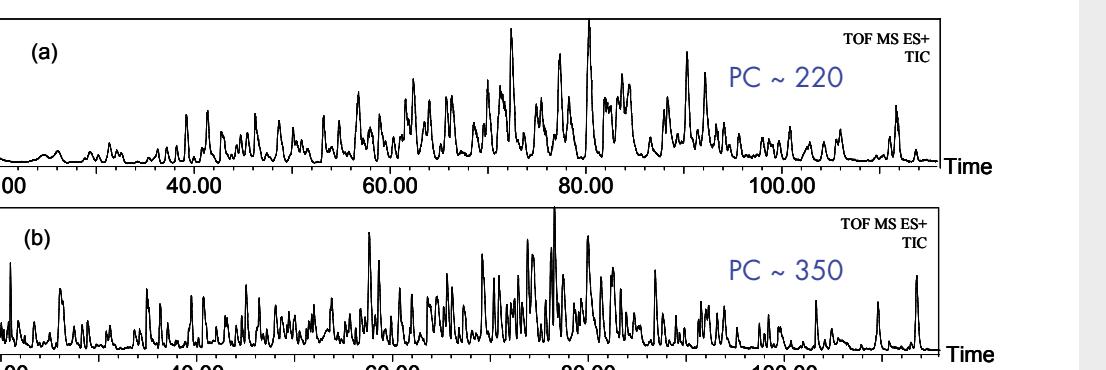


Figure 6. Column performance for 200 fmol 5-protein digest comparing 15 cm long columns packed with (a) 3 μm particles and (b) 1.7 μm particles, gradient: 3-40.6% B in 144 min.

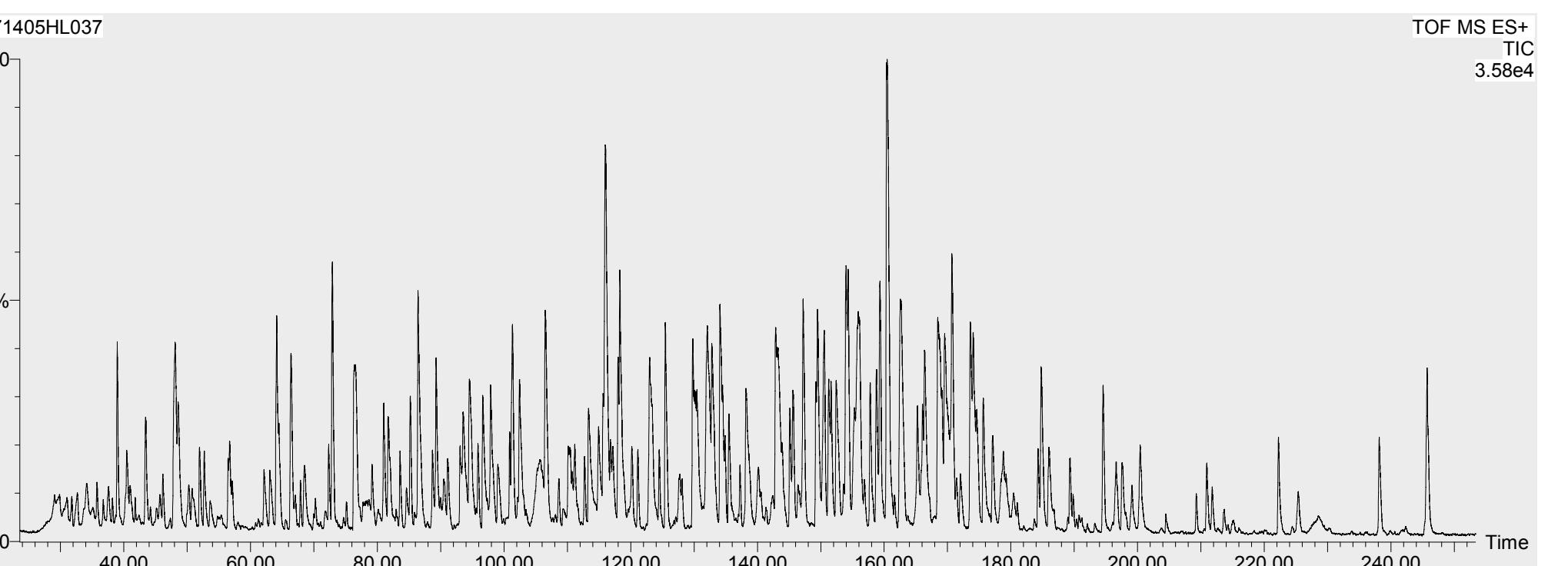


Figure 11. TIC from a nano UPLC separation of 200 fmol each of 5-protein digest for a 30 cm, 1.7 μm BEH column, gradient: 3-40.6% B in 336 min, column backpressure ~ 9,000. Peak capacity for this separation is ~ 550.

CONCLUSIONS

- The nanoACQUITY UPLC system is capable of generating accurate, reliable gradients at pressures approaching 10,000 psi, with good retention time reproducibility over extended periods of run time
- Using a 1.7 μm BEH vs. 3 μm particle for a 15 cm column with a 144 min gradient length results in a 59% increase in peak capacity
- For short gradient lengths (24 and 48 min), increasing column length does not significantly increase peak capacity for either particle
- The 15 cm, 1.7 μm BEH column yields greater peak capacity measured at the various gradient lengths than the significantly longer 50 cm column packed with 3 μm particles
- The 30 cm, 1.7 μm BEH column exhibits the greatest overall increase in peak capacity with gradient length
- There is a trade-off between increased separation efficiency and slight reduction in MS signal height with longer gradient lengths
- Combining the 1.7 μm BEH particles with longer column lengths and longer gradient lengths can provide greater information for nanoLC/MS/MS analysis of complex proteomic samples

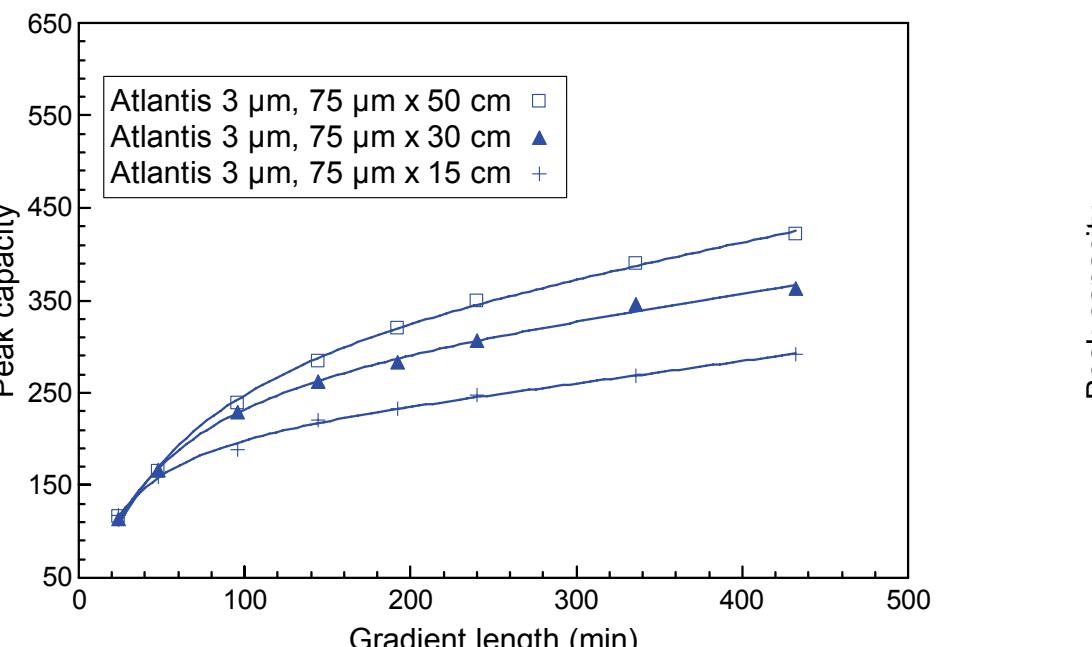


Figure 7. Gradient length vs. peak capacity plots for 15, 30, and 50 cm length columns packed with 3 μm Atlantis particles.

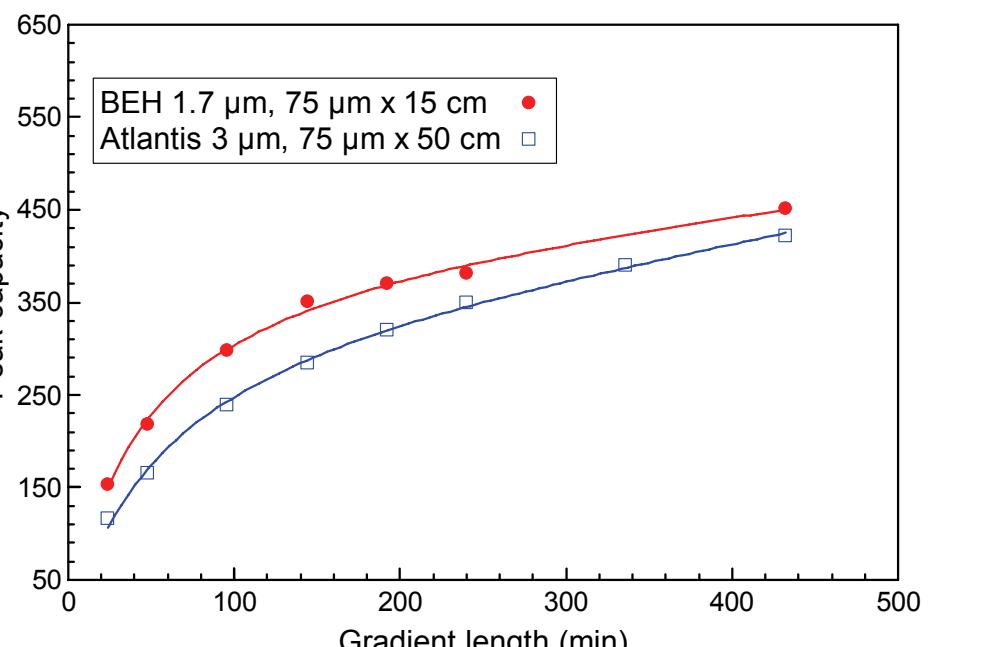


Figure 9. Gradient length vs. peak capacity plots comparing performance of a 15 cm, 1.7 μm BEH column with a 50 cm, 3 μm Atlantis column.

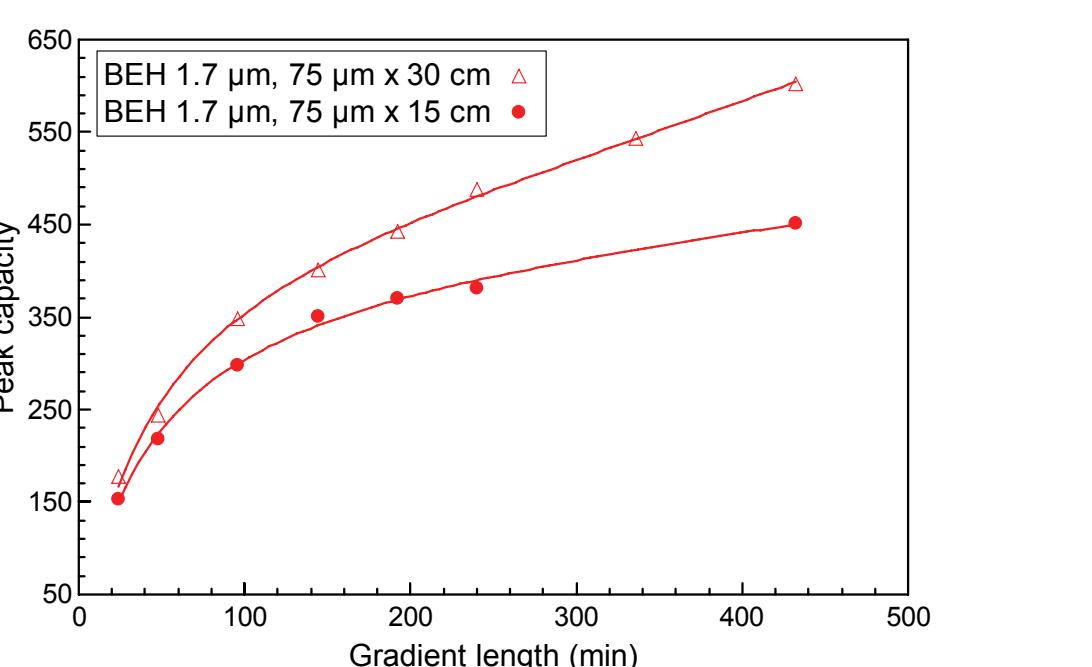


Figure 8. Gradient length vs. peak capacity plots for 15 and 30 cm length columns packed with 1.7 μm BEH particles.

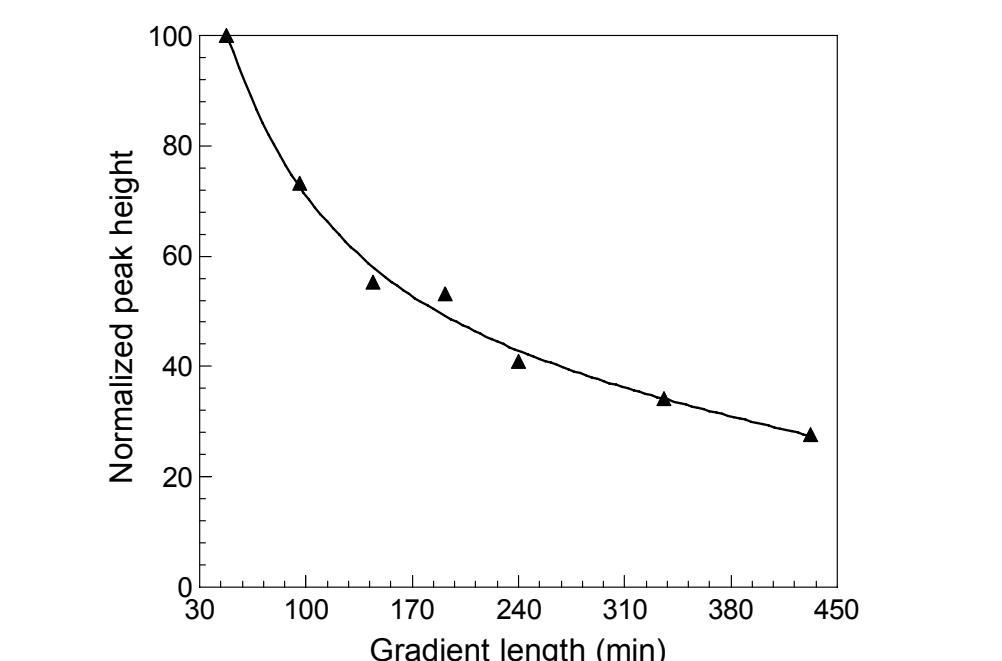


Figure 10. MS signal height vs. gradient length for 30 cm, 1.7 μm BEH particle column. The average MS signal height for 20 peptides eluting across the various gradient lengths was normalized to the average value obtained for the 48 min gradient.

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References

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